Journal of Medical Virology 59:135–140 (1999)

Identification of Hepatitis C Virus Seroconversion Resulting From Nosocomial Transmission on a Haemodialysis Unit: Implications for Infection Control and Laboratory Screening

Dianne N. Irish,[1]* Catherine Blake,[2] Jackie Christophers,[3] John E. Craske,[3] Lisa Burnapp,[4] Ian C. Abbs,[4] Eithne M.E. MacMahon,[1,5] Peter Muir,[5] Jangu E. Banatvala,[1,5] and Peter Simmonds[2]

[1]Department of Virology, Guy's and St. Thomas' Hospitals Trust, London, England
[2]Department of Medical Microbiology, University of Edinburgh, Edinburgh, Scotland
[3]Public Health Laboratory, Withington Hospital, Manchester, England
[4]Department of Renal Medicine, Guy's and St. Thomas' Hospitals Trust, London, England
[5]Department of Virology, The Guy's, King's College, and St. Thomas' Hospitals' Medical School, London, England

Hepatitis C virus (HCV) seroconversion was detected by routine screening in a haemodialysis patient, Patient 1. Serological investigations were undertaken over the following 3 months to determine if further transmission to other patients on the unit had occurred. No additional cases were identified. Twenty-two haemodialysis patients known to have HCV infection were investigated using molecular epidemiological methods to determine if transmission between patients had occurred. HCV viraemia was demonstrated by polymerase chain reaction in 19 of 22 patients (86%). Genotyping showed that eight patients were infected with genotype 1, three with genotype 3 and eight, including Patient 1, with genotype 2. Phylogenetic analysis of viral sequences from the eight patients with genotype 2 revealed three, including Patient 1,with a novel subtype of HCV type 2, and revealed close similarity between viral sequences from patient 1 and those from one other patient, suggesting transmission. This was consistent with haemodialysis histories. Among other patients with genotype 2, there were two with subtype 2a and three others with three separate novel subtypes, as yet undesignated. With the exception of patient 1, all patients infected with novel subtypes were of Afro-Caribbean origin. The HCV prevalence among patients on the haemodialysis unit was high (14%), which may reflect the ethnicity of our haemodialysis population. This case emphasises the risk of nosocomial transmission and the importance of infection control procedures on haemodialysis units, and highlights the usefulness of molecular epidemiological techniques for the investigation of outbreaks of HCV infection. ***J. Med. Virol. 59:135–140, 1999.***

KEY WORDS: HCV genotyping; subtypes; phylogenetic analysis; molecular epidemiology

INTRODUCTION

It is well established that outbreaks of hepatitis B virus (HBV) infection occurred in the early years of haemodialysis, associated with significant morbidity and mortality. In 1972, the Rosenheim Report made recommendations that decreased the incidence of HBV infections drastically among haemodialysis patients and staff, despite HBV vaccine being unavailable at that time [Department of Health and Social Security Advisory Group, 1972]. Although spread of HBV in haemodialysis units no longer poses such a threat, viruses recognised more recently such as hepatitis C virus (HCV) and the human immunodeficiency viruses (HIV-1 and HIV-2) are of concern.

HCV now accounts for the majority of parenteral and sporadic cases of non-A, non-B hepatitis worldwide. HCV seroprevalence is higher among haemodialysis patients than in the general population; reported prevalence rates in haemodialysis units around the world range from 3% in northern Europe [McIntyre et al., 1994; Schneeberger et al., 1998] to 71% in Kuwait [Kapoor et al., 1993]. The number of blood transfusions and duration of haemodialysis therapy are associated consistently with an increased risk of HCV infection in haemodialysis patients [Zeuzem et al., 1996]. In 1995, 6,500 patients were receiving maintenance haemodialysis in the United Kingdom, of whom 3,000 patients

*Correspondence to: Dr Dianne Irish, Department of Virology, Guy's and St. Thomas's Hospitals Trust, St. Thomas' Campus, Lambeth Palace Road, London SE1 7EH, England.

Accepted 18 December 1998

had developed end-stage renal failure and commenced haemodialysis that year [Berthoux, 1998]. This number represents a significant group at risk of HCV infection. However, little data are available on HCV prevalence in United Kingdom haemodialysis centres.

This report describes the case of a haemodialysis patient who seroconverted for HCV, the identification of the likely source of infection using molecular epidemiological methods, and the infection control issues involved. The report highlights the urgent need for updated national guidelines on control of blood-borne virus infections in the haemodialysis setting.

PATIENTS AND METHODS

Haemodialysis Units and Patients

In 1996, 150 adult haemodialysis patients were maintained on a three times weekly protocol at the Guy's and St. Thomas' Hospitals NHS Trust. There are two geographically distinct adult haemodialysis units, one at Guy's Hospital and the other at St. Thomas' Hospital. Twenty-one patients were known to have blood-borne virus infections, including 19 with HCV infection, 1 with HIV-1 and HBV infection, and 1 with HIV-1 infection. There were 16 stations on each unit, with two side rooms on the St. Thomas' site reserved for patients with HIV or HBV infection. At the Guy's site, patients with HIV or HBV infection received haemodialysis in side rooms on the renal wards. There were two shifts per day with three to four nurses per shift, with up to two qualified nurses per shift. Nursing staff were instructed in local guidelines for good practice, which included the practice of universal precautions [DHSS Advisory Report, 1972]. All new patients receiving haemodialysis were tested for HBV surface antigen (HBsAg), and HIV and HCV antibody. In addition, all haemodialysis patients were tested at 3-monthly intervals for HBV and HCV infection. General practitioners were asked to immunise the haemodialysis patients in their practice against hepatitis B infection. All haemodialysis machines were heat disinfected after use, and following haemodialysis of patients with blood-borne virus infections, an additional formalin disinfection was performed.

HCV Serology

Serum samples were tested for HCV antibody by the AxSYM third-generation microparticle enzyme immunoassay (EIA) (Abbott Laboratories, Abbott Park, IL). Reactive specimens were retested in duplicate. Repeatedly reactive samples were evaluated by HCV Recombinant Immunoblot Assay (RIBA, Chiron, Emeryville, CA), and in some cases by polymerase chain reaction (PCR) as described below. Units of transfused blood were screened by The South London Transfusion Centre, by Ortho EIA (Ortho Diagnostics Systems, Neckargemünd, Germany) and Sanofi EIA (Pasteur Laboratories, France).

HCV PCR

Serum samples were stored at ≤−20°C and tested for HCV RNA using HCV Amplicor Version 1.0 (Roche Diagnostic Systems Inc., Branchburg, NJ), according to manufacturer's instructions.

HCV Genotyping

HCV genotypes were identified by restriction fragment length polymorphism (RFLP) analysis of PCR products derived from the 5′ noncoding region (5′NCR) of the HCV genome as described previously [Chan et al., 1992; Davidson et al., 1995].

Phylogenetic Analysis

Phylogenetic analysis of nucleotide sequences was carried out on a 327-base pair fragment (positions 7941–8267) within the nonstructural gene, NS5 [Choo et al., 1991]. Sequences were compared using a distance based method (Jukes-Cantor distances at all sites followed by neighbour joining) as implemented on the MEGA package [Kumar et al., 1993]. Robustness of grouping was assessed by bootstrap re-sampling. Sequences compared included those of designated genotypes for which complete genomic sequences are available: HC-J6 (type 2a; [Okamoto et al., 1991]), HC-J8 (type 2b; [Okamoto et al., 1992]) and BEBE1 (type 2c; [Nakao et al., 1996]). Other partial length sequences in NS5 were K2a, K2b [Enomoto et al., 1990], FR5 (2a; [Stuyver et al., 1995]), NE91 (2b), CH333 (2c), CH563 (2c; [Stuyver et al., 1995]), NE92 (2d; [Vandoorn et al., 1994]), NL50 (2e), FR4 (2e; [Stuyver et al., 1995]), NL33 (2f; [Stuyver et al., 1995]), L48491, FR14, and FR15 (2l; [Stuyver et al., 1996a]) and the unassigned subtypes of type 2 predominantly found in West Africa, POL17, POL07, POL12, POL48, POL52 [Ruggieri et al., 1996]; HPCNS5BAB [Stuyver et al., 1996b] and FR18 [Stuyver et al., 1996a]; HN4 [Qu et al., 1994]; GUI30, BF235, BF247, BF70, BF108, BF229, BF307, BN89, BF191, BF230, BN85, BN177, BF201, BF4 [Jeannel et al., 1998]; and BA045, BA047, RU169, JK139, JK047, JK109, and JK025 [Tokita et al., 1998]. The sequence of HCV-PT [Choo et al., 1991] was used as an outgroup. The sequences obtained during this study have been deposited with GENBANK under the accession numbers AF164105–AF164112.

Ethnicity Data

Ethnicity data on haemodialysis patients were obtained by questionnaires or from a patient database.

RESULTS

Case Report

In May 1996, a 75-year-old male, Patient 1, was identified on routine serological screening to have seroconverted for HCV infection. Infection was confirmed by HCV RIBA and PCR. Stored sera from August and November 1995 were tested by PCR and were HCV-negative. He was asymptomatic, but during the period from November 1995 to May 1996, his alanine amino transferase (ALT) level peaked at 170 IU/ml, then returned to normal. He had received haemodialysis for end-stage renal failure for 15 months prior to diagnosis of HCV infection, and had received blood transfusions

in September 1995 and January 1996. No other risk factors for HCV infection were identified. The donors of the four units of transfused blood were recalled and retested, and all were seronegative for HCV. In view of the possibility of nosocomial transmission, infection control procedures and working practices of haemodialysis staff were reviewed. Serological screening and molecular methods were used to determine if further transmission had occurred, and to identify the most likely source of infection.

Determination of Spread of HCV Infection

Following the seroconversion incident, serological screening was undertaken monthly from June to August 1996 on all 150 haemodialysis patients, as well as continuous ambulatory peritoneal dialysis and transplanted patients who had received haemodialysis during the 6 months prior to the seroconversion of Patient 1, with the aim of identifying any further seroconversions. Two hundred patients were included in this study. In addition, ALT levels were monitored weekly.

No further cases of HCV infection were identified serologically, and no cases of unexplained ALT elevation were observed. Therefore, from September 1996, 3-monthly screening of haemodialysis patients resumed. However, in December 1996 a 30-year-old male patient, Patient 2, was found to have an HCV antibody result of 0.86 (assay cutoff = 1.0). Although within the negative range, this test reading was significantly higher than that of most seronegative samples. Because this patient had a history of two failed renal transplants, and because of the increased vigilance following the seroconversion incident, his serum was tested by RIBA and PCR. The sample was seronegative by RIBA, but HCV RNA positive by PCR. Stored sera from Patient 2 collected between October 1995 and October 1996 were tested and were HCV seronegative but positive for HCV RNA, indicating that he had been infected prior to Patient 1, and prior to his admission on to the unit in 1995. The National Look-Back Exercise [Department of Health, 1995] revealed that at the time of his second renal transplant in 1990, Patient 2 had been transfused with a unit of blood from an HCV-seropositive donor. Patient 2 seroconverted subsequently between February and August 1997, between 16 and 22 months after admission, and 7 years after the presumed time of infection.

In view of this new case, all 150 haemodialysis patients on the two units were screened for HCV infection in January 1997, but on this occasion by PCR. No further cases were identified.

Identification of the Source of Infection

When transmission of HCV infection by blood transfusion was ruled out in Patient 1, the possibility of nosocomial transmission was investigated. Specimens from 22 patients, including the 19 patients known to have HCV infection, Patient 1, Patient 2, and a further patient, Patient 3, who died in November 1995 but had received haemodialysis at the same time as Patient 1, were investigated by HCV genotyping, and phylogenetic analysis to identify possible transmission pathways. Of these 22 patients, viraemia was demonstrated by PCR in 19 patients. Genotyping was successful in all 19 HCV RNA-positive patients. Eight patients were found to be infected with genotype 1, three, including Patient 2, with genotype 3, and eight, including Patient 1, with genotype 2. Patients infected with genotype 2 were therefore investigated further by phylogenetic analysis of NS5 sequences. HCV NS5 sequences from three patients (Patient 1, Patient 3, and a third patient, Patient 4) shared more than 88% similarity, but only 75–86% similarity with currently recognised subtypes (Fig. 1). These sequences therefore correspond to a new subtype of HCV type 2 [Simmonds et al., 1993]. Of the other patients with genotype 2, two (patients 5 and 9) had subtype 2a and three (patients 6–8) had distinct novel subtypes (Fig. 1). With the exception of Patient 1, all patients with novel subtypes were Afro-Caribbean in origin.

Sequences of variants from patient 1 and patient 3 were related more closely to each other than to the variants infecting Patient 4 (Fig. 1). This finding is compatible with a transmission between patients 1 and 3. Examination of these patients' records revealed that Patient 4 had not been treated on the same unit as Patient 1, whereas Patient 3 had received haemodialysis at both units, and had been treated consistently on the same shift as Patient 1, without additional infection control precautions. The exact mode of transmission is unknown; the two patients were not treated in adjacent stations. Patient 1 was treated in a side room, whereas Patient 3 was treated on the open ward. However, a breakdown in infection control standards by healthcare personnel appears to be the most likely cause. Patient 3 had been diagnosed as HCV-infected in February 1995, and received haemodialysis from August to November 1995, 6–9 months prior to identification of seroconversion in Patient 1. Patient 3 died of cardiovascular disease in November 1995.

HCV Prevalence on the Haemodialysis Unit

At the conclusion of serological and molecular screening, the HCV prevalence among current haemodialysis patients at the Guy's and St. Thomas' sites was determined to be 14% (21/150).

HCV Infection and Ethnicity

We obtained ethnicity data on 87% of our current haemodialysis population. Of 137 patients, 72 (53%) were Caucasian, 36 (26%) were Afro-Caribbean, and 29 (22%) were Black African, Indian subcontinent, Pakistani, or Chinese. The prevalence of HCV infection was 11% (8/72) in Caucasians, 22% (8/36) in Afro-Caribbeans, and 17% (5/29) in the other ethnic groups. The difference in HCV prevalence between Caucasians

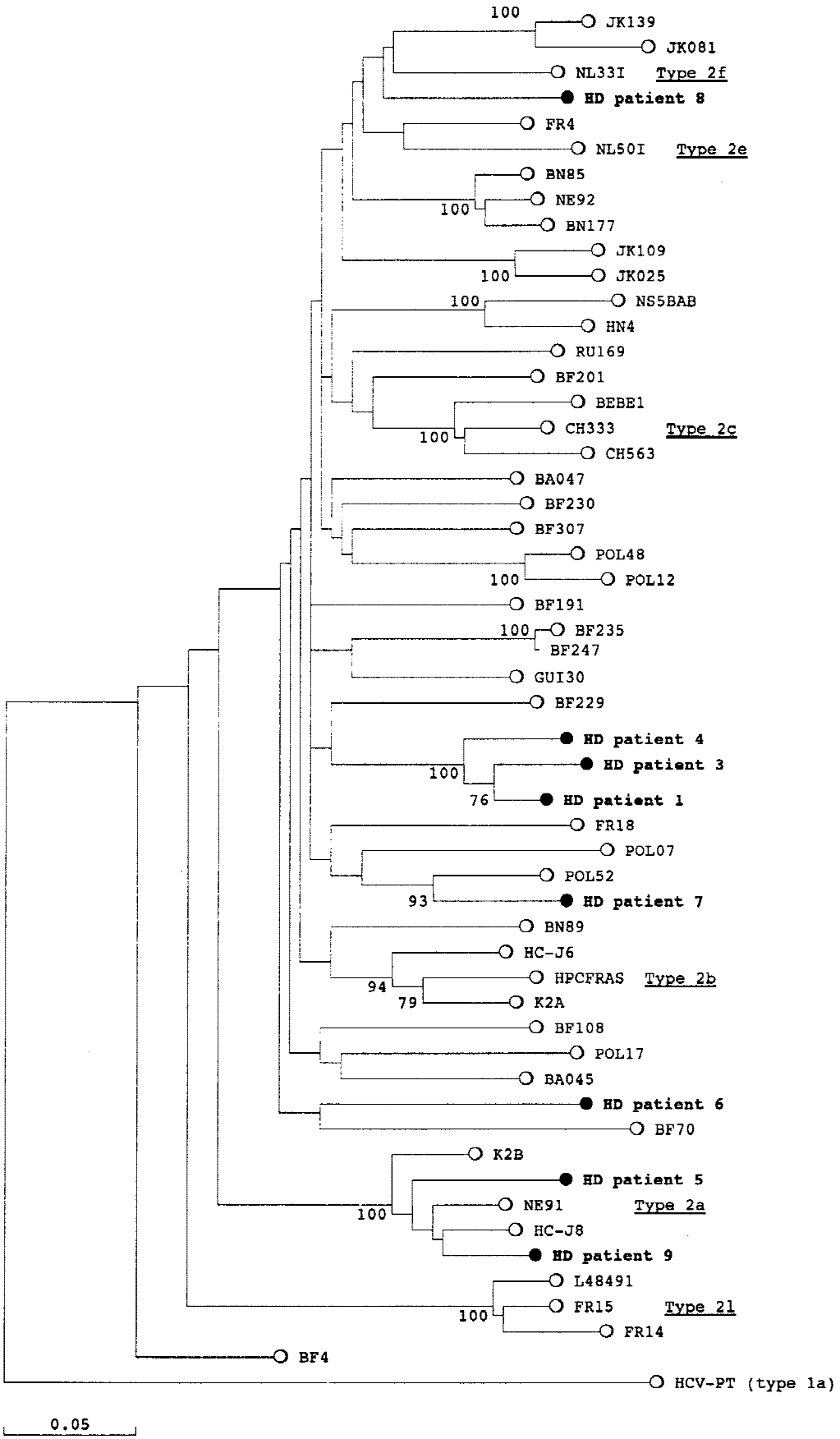

Fig. 1. Phylogenetic analysis of nucleotide sequences from the NS5 region from the eight study subjects infected with subtypes of hepatitis C virus (HCV) type 2 (haemodialysis patients 1, 3–9; represented as closed circles) and representative sequences from 30 other subtypes of type 2 (restricted to a maximum of three per subtype; represented as open circles). Numbers on branches indicate percentage of bootstrap samples supporting phylogeny (values of ≥75% only are shown). Evolutionary distance units are indicated in the scale.

and non-Caucasians was not statistically significant ($P = .1$; Fisher's exact test).

DISCUSSION

A nosocomially acquired HCV infection in a haemodialysis patient was documented, and the source of the infection was identified using molecular epidemiological methods. Phylogenetic analysis of HCV nucleotide sequences demonstrated close sequence similarity between the index case and one other patient, a result that was consistent with a transmission event between these patients. Phylogenetic analysis has been used to document historical transmissions among HCV-infected haemodialysis patients and staff [Allander et al., 1994; Munro et al., 1996; Zeuzem et al., 1996; Mizuno et al., 1998; Norder et al., 1998; Schneeberger et al., 1998]. The study illustrates how molecular methods can be used to pinpoint breakdowns in infection control procedures that result in nosocomial transmission. This procedure may be useful in formulating appropriate measures for containing outbreaks, and preventing future transmission.

In a recent study, Le Pogam et al. [1998] identified a rare HCV genotype in four haemodialysis patients who seroconverted. Only one other patient on the unit was infected with this genotype, and phylogenetic analysis confirmed that all four had acquired infection from this patient. In our case, infection was caused by HCV type 2, which was present in seven other haemodialysis patients. This study illustrates how transmission routes can also be identified using molecular methods when HCV infection is prevalent, and when common HCV genotypes are implicated.

Screening for elevations in ALT did not identify further cases in this study. Screening large numbers of patients by ALT may be useful and cost effective in outbreak investigations, since a rise in ALT may precede seroconversion [Le Pogam et al., 1998]. Those with an unexplained rise should be considered for testing by PCR, because this method may identify recently infected patients who have yet to seroconvert, as well as patients who fail to seroconvert. Seronegative HCV infection in haemodialysis patients has been reported previously [Bukh et al., 1993; Schneeberger et al., 1998] and this condition may be attributable to diminished immune responses. Patient 3 was on low dose maintenance steroids (5 mg prednisolone daily). However, haemodialysis may have also contributed to immune suppression [Khan and Catto, 1993].

We have also identified HCV infection in an HCV seronegative 10-year-old girl, a renal transplant recipient who had been treated previously on our paediatric haemodialysis unit, which is physically distinct from the adult units with separate staffing arrangements. She was identified as having HCV infection by PCR 3 months after admission to the haemodialysis unit. Retrospective analysis of stored sera revealed that she was HCV-infected but seronegative at the time of admission (results not shown). At follow-up 8 months later, this patient had seroconverted.

The 14% prevalence of HCV infection in our unit is higher than that reported from other centres in the United Kingdom [McIntyre et al, 1994] and northern Europe [Schneeberger et al., 1998], and is comparable with figures from some southern European and Mediterranean countries [Olmer et al., 1997]. This high prevalence may reflect the ethnic diversity of our inner city population. Indeed 20% of non-Caucasian patients in our group were HCV-infected, and several Afro-Caribbean patients were infected with previously unrecognised HCV subtypes, which may be representative of subtypes occurring in the Caribbean. The HCV prevalence among Caucasians in our haemodialysis population was also high; this group may include individuals of both northern and southern European origin. Although HCV transmission was identified in the haemodialysis unit, the diversity of genotypes and subtypes identified in our HCV-infected haemodialysis population suggested that frequent nosocomial transmission between patients did not occur.

Our unit is one of the largest in the United Kingdom, and our experience emphasises the importance of identifying patients with HCV infection and enforcing strict infection control measures to prevent transmission. Currently, guidelines for the management of HCV-infected patients in haemodialysis units are being formulated by a Public Health Laboratory Service Working Group: indeed, an update of the Rosenheim Report, providing additional guidelines on newly recognised blood-borne viruses, particularly HIV and HCV, is urgently required. In the absence of updated guidelines, we suggest the following recommendations:

1. With increasing numbers of patients receiving haemodialysis, standard infection control practices may be compromised. Under these circumstances, HCV-infected patients should be segregated while receiving haemodialysis where there are constraints on staffing and space. However, if resources are adequate, the practice of universal precautions may be sufficient and segregation of HCV-infected haemodialysis patients may not be necessary. Should new cases of HCV infection be identified, all HCV-infected patients should be segregated until the source of infection is identified, because these cases may have resulted from breakdown in infection control practices.
2. All new haemodialysis patients and transplant candidates should be screened for HCV infection by PCR and serology, bearing in mind that we identified two other patients who were HCV infected but seronegative at the time of admission, and remained seronegative for extended periods. The use of PCR for routine screening of haemodialysis patients is worthy of consideration, but requires further cost–benefit analysis.
3. Molecular methods should be used in the investigation and management of HCV outbreaks. This exercise highlights the usefulness of genotyping and phylogenetic analysis in identifying or excluding

nosocomial transmission, thus demonstrating where breakdowns in infection control measures have occurred.

ACKNOWLEDGMENTS

We thank Roche Diagnostics (United Kingdom) for providing HCV Amplicor kits for screening haemodialysis patients by PCR.

REFERENCES

Allander T, Medin C, Jacobson SH, Grillner L, Persson MAA. 1994. Hepatitis C transmission in a hemodialysis unit: molecular evidence for spread of virus among patients not sharing equipment. J Med Virol 43:415–419.

Berthoux F. 1998. Presented at the XXXVth Congress of European Renal Association/ European Dialysis and Transplant Association in Romany.

Berthoux F, Gellert R, Jones E, Mendel S, Valderrabano F, Briggs D, Carrera F, Cambi V, Saker L. 1998. Epidemiology and demography of treated end-stage renal failure in the elderly: from the European Renal Association (ERA-ADTA) Registry. Nephrol Dial Transplant 13(Suppl 7):65–68.

Bukh A, Wantzin P, Krogsgaard K, Knudsen T, Purcell RH, Miller RH, The Copenhagen Dialysis HCV Study Group. 1993. High prevalence of hepatitis C virus (HCV) RNA in dialysis patients: failure of commercially available antibody tests to identify a significant number of patients with HCV infection. J Infect Dis 168: 1343–1348.

Chan SW, McOmish F, Holmes EC, Dow B, Peutherer JF, Follett E, Yap PL, Simmonds P. 1992. Analysis of a new hepatitis C virus type and its phylogenetic relationship to existing variants. J Gen Virol 73:1131–1141.

Choo QL, Richman KH, Han JH, Berger K, Lee C, Dong C, Gallegos C, Coit D, Medina Selby R, Barr PJ, Weiner AJ, Bradley DW, Kuo G, Houghton M. 1991. Genetic organization and diversity of the hepatitis C virus. Proc Natl Acad Sci USA 88:2451–2455.

Davidson F, Simmonds P, Ferguson JC, Jarvis LM, Dow BC, Follett EAC, Seed CRG, Krusius T, Lin C, Medgyesi, Kiyokawa H, Olim G, Duraisamy G, Cuypers T, Saeed AA, Teo D, Conradie J, Kew MC, Lin M, Nuchaprayoon C, Ndimbie OK, Yap PL. 1995. Survey of major genotypes and subtypes of hepatitis C virus using RFLP of sequences amplified from the 5′non-coding region. J Gen Virol 76:1197–1204.

Department of Health. 1995. Hepatitis C and blood transfusion look back. London: HMSO, PL CMO(95)1.

Department of Health and Social Security Advisory Group. 1972. Hepatitis and the treatment of chronic renal failure. London: HMSO.

Enomoto N, Takada A, Nakao T, Date T. 1990. There are two major types of hepatitis C virus in Japan. Biochem Biophys Res Commun 170:1021–1025.

Jeannel D, Fretz C, Traore Y, Kohaemodialysisjo N, Bigot A, Gamy EP, Jourdan G, Kourouma K, Maertens G, Fumoux F, Fournel JJ, Stuyver L. 1998. Evidence for high genetic diversity and long-term endemicity of hepatitis C virus genotypes 1 and 2 in West Africa. J Med Virol 55:92–97.

Kapoor M, el-Rashaid K, al-Mufti S, Sanad NA, Koshy A. 1993. Is dialysis environment more important than blood transfusion in transmission of Hepatitis C virus during haemodialysis? Vox Sanguinis 65:331.

Khan IH, Catto GR. 1993. Long term complications of dialysis infection. Kidney Int Suppl 41:S143–S148.

Kumar S, Tamura K, Nei M. 1993. MEGA: molecular evolutionary genetics analysis. version 1.0. University Park, PA: Pennsylvania State University.

Le Pogam S, Le Chapois D, Christen R, Dubois F, Barin F, Goudeau A. 1998. Hepatitis C in a haemodialysis unit: molecular evidence for nosocomial transmission. J Clin Microbiol 36:3040–3043.

McIntyre PG, McCruden EA, Dow BC, Cameron SO, McMillan MA, Allison ME, Briggs JD. 1994. Hepatitis C virus infection in renal dialysis patients in Glasgow. Nephrol Dial Transplant 9:291–295.

Mizuno M, Higuchi T, Kanmatsuse K, Esumi M. 1998. Genetic and serological evidence for multiple instances of unrecognised transmission of hepatitis C virus in hemodialysis units. J Clin Microbiol 36:2926–2931.

Munro J, Douglas Briggs J, McCruden EAB. 1996. Detection of a cluster of Hepatitis C infections in a renal transplant unit by analysis of sequence variation of the NS5a Gene. J Infect Dis 174: 177–180.

Nakao H, Okamoto H, Tokita H, Inoue T, Iizuka H, Pozzato G, Mishiro S. 1996. Full-length genomic sequence of a hepatitis C virus genotype 2c isolate (BEBE1) and the 2c-specific PCR primers. Arch Virol 141:701–704.

Norder H, Bergström Å, Uhnoo I, Aldén J, Weiss L, Czajkowski J, Magnius L. 1998. Confirmation of nosocomial transmission of hepatitis C virus by phylogenetic analysis of the NS5-B region. J Clin Microbiol 36:3066–3069.

Okamoto H, Kurai K, Okada S, Yamamoto K, Lizuka H, Tanaka T, Fukuda S, Tsuda F, Mishiro S. 1992. Full-length sequence of a hepatitis C virus genome having poor homology to reported isolates: comparative study of four distinct genotypes. Virology 188: 331–341.

Okamoto H, Okada S, Sugiyama Y, Kurai K, Iizuka H, Machida A, Miyakawa Y, Mayumi M. 1991. Nucleotide sequence of the genomic RNA of hepatitis C virus isolated from a human carrier: comparison with reported isolates for conserved and divergent regions. J Gen Virol 72:2697–2704.

Olmer M, Bouchouareb D, Zandotti C, De Micco P, De Lamballerie X. 1997. Transmission of the hepatitis C in a hemodialysis unit: evidence for a nosocomial transmission. Clin Nephrol 47:263–270.

Qu D, Hantz O, Gouy M, Vitvitski L, Li JS, Berby F, Tong SP, Trepo C. 1994. Heterogeneity of hepatitis C virus genotypes in France. J Gen Virol 75:1063–1070.

Ruggieri A, Argentini C, Kourama F, Chionne P, Dugo E, Spada E, Dettori S, Sabbatani S, Rapicetta M. 1996. Heterogeneity of hepatitis C virus genotype 2 variants in West Central Africa (Guinea Conakry). J Gen Virol 77:2073–2076.

Schneeberger PM, Keur I, van der Vliet W, van Hoek K, Boswijk H, van Loon A, van Dijk WC, Kauffmann RH, Quint W, van Doorn L-J. 1998. Hepatitis C virus infections in dialysis centers in the Netherlands: a national survey by serological and molecular methods. J Clin Microbiol 36:1711–1715.

Simmonds P, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL, Kolberg J, Urdea MS. 1993. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS5 region. J Gen Virol 74:2391–2399.

Stuyver L, Esquivel C, Boudifa A, Jaulmes D, Azar N, Lunel F, LerouxRoels G, Maertens G, Fournel JJ. 1996a. Hepatitis C virus (HCV) genotype analysis in apparently healthy anti-HCV-positive Parisian blood donors. Transfusion 36:552–558.

Stuyver L, Wyseur A, Vanarnhem W, Hernandez F, Maertens G. 1996b. Second-generation line probe assay for hepatitis C virus genotyping. J Clin Microbiol 34:2259–2266.

Stuyver L, Wyseur A, Vanarnhem W, Lunel P, Laurentpuig P, Pawlotsky JM, Kleter B, Bassit L, Nkengasong J, Vandoorn LJ, Maertens G. 1995. Hepatitis C virus genotyping by means of 5′-UR/core line probe assays and molecular analysis of untypeable. Virus Res 38:137–157.

Tokita H, Okamoto H, Iizuka H, Kishimoto J, Tsuda F, Miyakawa Y, Mayumi M. 1998. The entire nucleotide sequences of three hepatitis C virus isolates in genetic groups 7–9 and comparison with those in the other eight genetic groups. J Gen Virol 79:1847–1857.

Vandoorn LJ, Kleter B, Stuyver L, Maertens G, Brouwer H, Schalm S, Heijtink R, Quint W. 1994. Analysis of hepatitis C virus genotypes by a line probe assay and correlation with antibody profiles. J Hepatol 21:122–129.

Zeuzem S, Scheuermann EH, Waschk J, Lee J-H, Blaser C, Franke A, Roth KW. 1996. Phylogenetic analysis of hepatitis C virus isolates from hemodialysis patients. Kidney Int 49:896–902.